Preliminary Evaluation of Factors Affecting Perchlorate Uptake, Accumulation, and Redistribution in Lettuce

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Summary

The Colorado River is contaminated with low levels of perchlorate associated with aerospace and defense related industries near the Las Vegas Wash. Perchlorate, and other anions, have the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter (NIS). There is concern that this perchlorate-contaminated water may represent a health risk both as source of drinking water to humans and irrigation water to food crops. Over 95% of the lettuce consumed during the winter months in the United States is produced in the Lower Colorado River region of California and Arizona and there is concern this may further enhance exposure to perchlorate. The objective of this project is to perform a preliminary evaluation of factors affecting perchlorate uptake, accumulation, and redistribution in lettuce. Total uptake of perchlorate in the above ground plant of iceberg lettuce was approximately 3 g/ha. The results of the uptake study and the survey indicate most of the perchlorate accumulated in the outer frame and wrapper leaves and not the edible head. Although anion competition, including fertilization, can sometimes affect perchlorate accumulation, the differences were small in magnitude and of negligiable practical importance.

Introduction

Perchlorate has been discovered in surface and ground water supplies throughout the United States. There is concern that these perchlorate-contaminated waters may represent a health risk both as sources of drinking water to humans and irrigation water to food crops. Perchlorate has the potential to cause thyroid dysfunction by inhibiting iodide uptake by the sodium iodide symporter (NIS) (Clark, 2000).

Perchlorate contamination of the Colorado River is well documented and the concentration at Lake Mead had been measured as high as 14 ppb. This contamination is introduced into Lake Mead through contamination by a perchlorate salt manufacturing plant associated with aerospace and munitions industries on the Las Vegas Wash. It has been reported that the Colorado River below Lake Mead has concentrations ranging from 5 to 9 ppb (DHS, 2000). The production of fresh market vegetables in the lower Colorado regions of Arizona and California is a 2 billion dollar industry. Essentially 100% of this industry relies on Colorado River water for irrigation and there is concern that consumption of perchlorate through food produced in the region may represent another significant source of exposure.

Several plant species have been shown to absorb and accumulate perchlorate from soil and irrigation water. Urbansky et al., (2000) found perchlorate uptake and accumulation by salt cedar

(*Tamarix ramosissima*) growing in the Las Vegas wash. Accumulation of perchlorate in tobacco fields previously fertilized with perchlorate containing Chilean nitrate is also well established (Ellington et al., 2001). Accumulation by Salix (Nzengung et al., 1999; Nzengung and Wang, 2000) and Myriophyllum aquaticum (Susarla, 1999) has been studied for phytoremdiation.

There is also evidence that perchlorate accumulates in certain food crops. This was initially inferred from studies with pertechnetate. Pertechnetate is chemically similar to perchlorate and is often considered an analog for the study of perchlorate. Numerous studies have shown pertechnetate is absorbed and accumulates in leaves but not to the same extent in the edible portions of fruiting plants (Gast et al., 1978; Cataldo et al., 1986; Echevarria et al., 1997).

Accumulation in crops where leaves are consumed is a major concern. Hutchinson et al., (2000) found perchlorate accumulated in lettuce during early growth stages under conditions in the glasshouse. In a more recent glasshouse experiment, Yu et al., (2003) evaluated perchlorate uptake by lettuce, cucumber, and soybean in sand culture. Perchlorate accumulation was higher in lettuce compared to cucumber and soybeans. At the request of the Environmental Working Group, The Institute of Environmental and Human Health of Texas Tech University analyzed 22 leafy vegetable samples purchased in Northern California in the winter of 2002-2003 (EWG, 2003). Presumably these samples were from the lower Colorado River region since they were purchased in the winter months when most of the leafy produce is shipped from this region. Four of the 22 samples contained detectable levels of perchlorate, one as high as 121 ug/kg on a fresh weight basis. More recently, the FDA in a preliminary “bread basket” survey found perchlorate in lettuce irrigated with Colorado River water to range from below quantifiable levels to 129 ug/L (FDA, 2004).

It is likely that competing anions affect the uptake perchlorate (or its analogs) by plants. Pertechnetate absorption and accumulation by plants is affected by other anions including nitrate (Echevarria et al., 1998; Krijger 2000), sulfate (Cataldo et al., 1978; 1983) and phosphate (Cataldo et al., 1978; 1983; Echevarria et al., 1997; 1998). It has been shown that nitrate competitively inhibits chlorate uptake (Deanne-Drummond and Glass, 1982). Nzengung et al., (1999) found nitrate concentrations greater than 400 mg/L prevented the removal of perchlorate from solution culture by willow seedings. Yu et al., (2003) found reduced perchlorate uptake in cucumber as the ratio of nutrient solution to water was increased, presumably due to anion competition by nitrate. Colorado River water contains approximately 250 mg/L sulfate, 180 mg/L bicarbonate 90 mg/L chloride, 0.4 mg/L fluoride, and 0.2 mg/L nitrate. We had no information to what extent anion competition might reduce perchlorate uptake under natural conditions in the lower Colorado River basin.

Fertilizer is sometimes considered a potential source of perchlorate to plants. Chilean nitrate has long been known to be a natural source of perchlorate (Ericksen, 1983). A recent comprehensive evaluation has shown that with the exception of fertilizers derived from Chilean nitrate, fertilizers are not a significant source of perchlorate to the environment (Urbansky et al., 2001). A limited amount of Chilean nitrate has been used in vegetable, fruit, and tobacco production systems. More recently it was frequently used in organic production systems, as it was the only form of mineral N allowed. However, it is estimated less the 0.1% of the N fertilizer used in the United States is derived from Chilean nitrate. A recent survey by the USGS has found perchlorate in other agricultural amendments (Orris et al., 2003) including blood meal, fishmeal, and kelp. Perchlorate has also been identified in evaporate mineral deposits in the southwestern United States (Orris and Harvey, 2004). It is likely irrigation water is the major source of perchlorate in lettuce produced in the lower Colorado River region. However, because perchlorate can sometimes occur in some fertilizers, amendments, and some natural evaporates, other sources cannot completely be ruled out. More work is needed to identify all potential natural and anthropogenic sources of perchlorate in the environment.

Over 60,000 ha of lettuce are produced in the lower Colorado River region of Arizona and California each year. Lettuce is seeded from early September through late January and harvested from early November through early April each year. The confluence of the Colorado and Gila Rivers occurs north of the international border in Yuma County, Arizona. Lettuce is produced in the former alluvial flood plains of the Colorado and Gila River Valleys as well as the Coachella and Imperial Valleys of California. The Imperial and Coachella Valleys of California are irrigated with Colorado River water diverted into the "All American Canal" at the Imperial Diversion Dam. Production along the Gila River including the North and South Gila Valley and the Wellton-Mohawk Irrigation and Drainage District are also irrigated with Colorado River water transported in canals from the Imperial Diversion Dam.

The objective of this project is to perform a preliminary evaluation of factors affecting perchlorate uptake, accumulation, and redistribution in lettuce.

Materials and Methods

<u>Uptake and accumulation of perchlorate in iceberg lettuce</u>

All fields sampled were irrigated with Colorado River water. During 2003-2004 samples were collected from one location to study uptake and distribution of perchlorate in iceberg lettuce during the growing season. This evaluation was located on a site in Yuma seeded October 7, 2003. Four replicate samples were collected at key growth stages throughout the growing season. The number of plant samples composited into each replicate varied by plant age and size and ranged from 400 plants at the two-leaf growth stage to two plants at maturity. The 1-2 leaf, 6-7 leaf, folding, heading, and maturity growth stages occurred 8, 24, 43, 54, and 100 days after planting, respectively. For each replicate on each sampling data one set of plants was separated into whole above ground plants and roots. Another set was collected for more detailed partitioning. As the plant developed this second set was partitioned from the outer leaves inward. For example, as the plant grew we separated the outer four leaves (1-4L), the next four inner leaves (5-8L), the next four inner leaves (9-12L), and the core or head. The weights of each portion were recorded. These samples were then diced, mixed thoroughly, and a sub-sample was placed in the freezer.

In the 2002-2003 winter-spring season, fields in the Lower Colorado River Valley of Arizona (Yuma), the Gila Valley (North and South) and the Wellton-Mohawk Irrigation and Drainage District were sampled in February. These would represent fields seeded in November. In the 2003-2004 fall-winter season, fields in these same areas were sampled November through January. These would represent fields seeded in September and early October. After recording the location, we took seven whole plants at random from each 40-acre field sampled and transported them to our laboratory. Three plants were processed as whole above ground plants. Four plants were partitioned into wrapper and frame leaves and trimmed naked edible heads. The weights of each portion were recorded. These samples were then diced, mixed thoroughly, and a sub-sample was placed in the freezer.

<u>Greenhouse N Study</u>
A greenhouse study was conducted to evaluate the effect of N fertilizer rate and source on perchlorate uptake by lettuce. This study used 1.5 L pots with an inside diameter 15 cm and each filled with approximately 1.5 kg of soil. Phosphorus was applied at 0.35 g/pot as monocalcium phosphate. In this experiment, lettuce was irrigated with Colorado River water spiked with perchlorate to a final concentration of 20 ug/L. The N sources were urea, ammonium nitrate, calcium nitrate, and ammonium sulfate. Fertilizers were added to achieve N rates of 0, 0.35, 0.70, and 1.4 g N pot. These rates approximately corresponded to field N rates of 0, 200, 400, and 800 kg/ha based on surface area. These N rates were split into four applications during the growing period. Because of the difficulties of growing iceberg lettuce in the greenhouse we used a leaf lettuce cultivar 'Ventana' in this experiment. The experimental design was randomized complete block with four replications. The lettuce was transplanted into pots October 21, 2003. This experiment was harvested December 5 and above-ground plant material and roots were stored in the freezer.

<u>Greenhouse P experiment</u>
A greenhouse study was conducted to evaluate the effect of P fertilizer rate on perchlorate uptake by lettuce. This study used 1.5 L pots with an outside diameter of 15 cm each filled with approximately 1.5 kg of soil. N was applied to all pots as ammonium nitrate split in four equal applications over the growing period. In this experiment, lettuce was irrigated with Colorado River water spiked with perchlorate to a final concentration of 20 ug/L. The P rates were 0.17, 0.35, and 0.7 g/pot as monocalcium phosphate. Because of the difficulties of growing iceberg lettuce in the greenhouse we used a leaf lettuce cultivar 'Ventana' in this experiment. The experimental design was randomized complete block with four replications. The lettuce was transplanted into the pots October 20, 2003. This experiment was harvested December 12 and above-ground plant material and roots were stored in the freezer.

<u>Greenhouse anion competition study</u>
This study was conducted to evaluate the effect of ion concentration in the irrigation water, as measured by conductance, on perchlorate uptake and accumulation by lettuce. This study used 1.5 L pots with an outside diameter of 15 cm each filled with approximately 1.5 kg of soil. N was applied to all pots as ammonium nitrate split in four equal applications over the growing period. P was applied at 0.35 g/pot before transplanting. The treatments included Colorado River (CRW) water diluted with DI water or concentrated by evaporation to achieve various levels of conductance. The treatments included 6.25% CRW, 12.5% CRW, 25% CRW, 50% CRW diluted with 93.75%, 87.5%, 75%, and 50% DI water, respectively. In addition we had treatments consisting of 100% CRW and 200% obtained by evaporation. All irrigation water treatments were spiked to a final perchlorate concentration of 20 ug/L. Because of the difficulties of growing iceberg lettuce in the greenhouse we used a leaf lettuce cultivar 'Ventana' in this experiment. The experimental design was randomized complete block with five replications.

The lettuce was transplanted into the pots November 4, 2003. This experiment was harvested January 8, 2004 and above-ground plant material and roots were stored in the freezer.

<u>Field N experiment#1</u>
This experiment was an N rate study. Nitrogen fertilizer rates were 0, 50, 100, 150, 200, and 250 kg/ha as ammonium nitrate. These N rates were applied in two equal sidedress applications. The experimental design was a randomized complete block with three replications. Lettuce ‘Red Coach 74’ was seeded October 8, 2001 and harvested January 22, 2002. Lettuce was irrigated with Colorado River water containing ambient levels of perchlorate. Lettuce plant samples were oven-dried and stored until analysis.

<u>Field N experiment#2</u>
This field experiment evaluated N rate an N source study. The nitrogen sources were urea, ammonium nitrate, calcium nitrate, and ammonium sulfate. The N rates were 0, 100, and 200 kg/ha. The experimental design was randomized complete block with four replications. Lettuce ‘Coach Supreme’ was seeded November 18, 2003 and harvested March 18, 2004. Lettuce was irrigated with Colorado River water containing ambient levels of perchlorate. Lettuce plant samples were oven-dried and stored until analysis.

<u>Field P experiment</u>
This field experiment evaluated P rate and source. The P sources were ammonium polyphosphate and phosphoric acid. The P rates were 0, 50, 100, and 150 kg/ha. The experimental design was randomized complete block with four replications. Lettuce ‘Coach Supreme’ was seeded November 18, 2003 and harvested March 18, 2004. Lettuce was irrigated with Colorado River water containing ambient levels of perchlorate. Lettuce plant samples were oven-dried and stored until analysis.

<u>Sample processing and extraction for perchlorate</u>
The frozen samples were freeze-dried as space became available on a Labconco Model freeze drier. Weights before and after freeze-drying were recorded. Lettuce typically took 48 hours for complete freeze-drying. The samples were ground and stored in vials for extraction.

Because we did not have the freezer storage and freeze-drying capacity to handle all the volume of tissue samples collected in the field fertilizer experiments, tissue collected in the field experiments was oven-dried. A preliminary comparison of freeze drying and oven drying on split samples showed similar results.

We used an extraction procedure described previously (Ellington and Evans, 2000) with minor modifications. Briefly, 600 mg of freeze-dried product was weighed out into centrifuge tubes and 15 mL of DI water were added. The tubes were boiled for 30 minutes and the contents were placed in a refrigerator overnight with occasional gentle shaking. The tubes were then centrifuged for 30 minutes and the supernatants filtered through Kim wipes and filtered through 0.2 um Gel man ion membrane syringe filters. This solution was stored in vials labeled “extract one”. Two mL of the above extract (extract one) was reacted with 1000 mg DD-alumina overnight in vials. Vials were gently agitated two or three times over a 24-hour period. Eighteen mL if DI water were then added to this mixture. After stirring and settling, this solution was filtered through another 0.2 um Gel man ion membrane syringe filter and the resulting solution was labeled "extract 2". This sample was stored in the freezer until analysis by ion chromatography (IC). Before loading on IC this extract was allowed to reach room temperature and was filtered through a Dionex "On Guard" RP syringe filter. The On Guard filters had been pre-cleaned first with methanol then with DI water. Furthermore, the first 0.75 mL of sample (extract 2) pushed through the filter is discarded and the remaining aliquots used for IC analysis.

Perchlorate Analysis

All perchlorate analysis were performed on a Dionex 2500 located at the Soil, Water, and Environmental Science Research Laboratory at the University of Arizona's Yuma Agricultural Center. The Dionex 2500 contains an IP 25 isocratic pump, an EG50 eluent generator, a continuous regenerating trap column, a CD conductivity detector, the 2 mm AG16/AS16 guard and separation column pair, and an AMMS III suppressor. The columns, suppressor, and detector are housed in a LC 30 chromatography oven. We used a 50 mM KOH eluent and 50 mM sulfuric acid suppression. A 1000 uL injection loop was used and elution time ranged from 9.5 to 11.0 minutes. Calibration was performed with standards ranging from 0.5 to 100 ug/L. The coefficient of determination was greater than 0.99. Ideally, one should calibrate in matrix, but this is difficult to do for environmental and biological specimens because matrices are not constant. Therefore, we guarded against matrix errors by spiked additions. A minimum of 10% of the lettuce samples were extracted with a 100 ug/L perchlorate standard to yield 10 ug/L perchlorate after dilution. The method detection limit (MDL) was determined using the procedure outlined in EPA method 314.0 (USEAP, 1999) using seven replicates of a standard in reagent water. The calculated MDL was 0.2 ug/L using a 0.5 ug/L standard. We set the MRL for lettuce extracts at 1.5 ug/L. As a standard practice we would run 10% duplicate extractions in addition to the 10% spiked additions. Duplicate aliquots of a given extraction were always analyzed. Additional aliquots or replicates were analyzed if we judged variability on the first two aliquots or replicates excessive. In addition, duplicate extracts for approximately 10% of all samples were sent out to a commercial laboratory for confirmatory analysis by LC/MS/MS.

Perchlorate concentration in irrigation water

In addition to plant material we obtained sub-samples of composite of Colorado River water samples collected by the U.S. Bureau of Reclamation (USBOR). The USBOR and the International Boundary Commission collect these samples for water quality assessments approximately twice monthly. These water samples were analyzed for perchlorate in our laboratory using a modification of EPA Method 314.0. These samples were collected from March 2003 through April 2004. These measurements were compared to samples collected up-stream at Willow Beach by the Nevada Division of Environmental Protection from December 2002 through April 2004. All water samples were analyzed in our laboratory filtered through a combination of filters including "On guard Ba", "On Guard Ag", "On Guard H" and a 0.45 u membrane syringe filter. This combination was intended to reduce sulfate, chloride, and bicarbonate backgrounds as well as particulate matter and colloidal Ag. The first 3 mL of sample pushed through the filter series was discarded and the remaining aliquots used for IC analysis.

Results and Discussion

Perchlorate concentrations in the Colorado River over the lettuce production period of these surveys ranged from 1.5 to 8 ug/L (Figure 2). Perchlorate concentrations of water samples collected at Willow Beach 11 miles downstream of Lake Mead generally correspond closely to those we measured at Imperial Diversion Dam 290 miles downstream of Lake Mead. There were a couple of exceptions. For example, at Imperial Dam we did not observe the spike in concentration measured at Willow Beach in January and February of 2004. Conversely, at Imperial Dam we measured a spike in concentration in April 2004 that was observed at Willow Beach. Diversions, storage, and tributaries in between Willow Beach and Imperial Dam would likely cause some differences in concentrations of perchlorate measured at these sampling points. Overall, these values for perchlorate concentration generally agree with values determined from periodic grab samples collected by the Arizona Department of Environmental Quality (ADEQ, 2004).

Studies have shown that perchlorate in soils is largely depositional (Urbansky and Brown 2003; Khandaker and Sanchez, 2005). For the situation in the lower Colorado Region, perchlorate is transported into and through soils with irrigation water with no physical or chemical retention by the soils. It is possible for perchlorate to temporarily accumulate in the crop-rooting zone when evapotranspiration (ET) exceeds leaching. Nevertheless, because lettuce is salt sensitive, growers typically apply irrigation water to achieve leaching fractions to preclude detrimental salt accumulation. Therefore, over a growing period the perchlorate concentration of the irrigation water is a reasonable estimate of potential plant availability.

The average concentration of perchlorate in irrigation water over the survey period close was 5 ug/L. Lettuce typically receives 50 cm of irrigation water and we would therefore estimate total perchlorate applied to a lettuce crop in irrigation water to be approximately 25 g/ha. However, this would include the water applied for leaching salts as well. It should be noted that this water leaching below the roots zone is a potential source of contamination to drainage systems and ground water and additional work to address this potential impact is needed. A better estimate of potential perchlorate accumulation might be obtained from ET estimates for lettuce, which is approximately 25 cm (Erie et al., 1982; Martin et al, 1999), giving a potential perchlorate accumulation of 12 to 13 g/ha.

Actual estimates of perchlorate concentration and accumulation by iceberg lettuce over a growing season are shown in Table 1. Interestingly, perchlorate accumulation was highest in the outer leaves and decreased as the lettuce was partitioned inward toward the edible core. It is likely that perchlorate moves into plants in the transpiration stream and it accumulates as water transpires through the leaves (Ellington et al., 2001; Sunberg et al., 2003). For iceberg lettuce, transpiration largely occurs in the outer leaves. The results of a survey with iceberg lettuce corroborate these findings indicating most of the perchlorate accumulated in the outer frame and wrapper leaves and not the edible head (Table 2). Perchlorate concentrations in the total above ground plant ranged from below quantifiable levels to 142 ug/kg fw while concentrations in the frame and wrapper leaves ranged from below quantifiable levels to 195 ug/kg fw. Amounts in the edible head ranged from below detection to 26 ug/kg fw. The frame leaves are typically left in the field after harvesting and the grocer and/or consumer trim the wrapper leaves. The edible core represents the portion typically consumed.

These data show total above ground perchlorate accumulation for iceberg lettuce to be approximately 3 g/ha (Table 1). This value is consistent with amounts calculated from other data in this report. The reasons for total perchlorate accumulation being less than estimated potential accumulation are unknown. It may be due to microbiology-mediated reduction of perchlorate in soil water at anaerobic micro-sites in the soil (Coates et al., 2000), it may be associated with some plant selectivity rather than purely passive uptake, or it may be biochemical reduction in the plant (Nzengung et al. 1999). Additional work is needed to understand factors and mechanisms affecting plant uptake, transport, and transformations of perchlorate.

The effects of N fertilization on plant growth and perchlorate accumulation in the greenhouse experiment are shown in Table 3. Data are presented as main effect means. Lettuce dry matter production varied by N rate. The significant quadratic trend reflects the increase to the first increment of N and a decrease at the highest increment of N. Yield reduction at the high N rate was more pronounced with the urea N source. Perchlorate concentration declined with the first increment of N but total uptake increased suggesting this observed differences was largely due to growth dilution rather than anion competition. Although perchlorate concentration and uptake did decline at the high rate of N, this rate was toxic to plant growth and would not be used under normal production practices.

The effects of N fertilization on lettuce perchlorate accumulation was also evaluated under field conditions. In the first field experiment, while yield and N accumulation increased to N rate there were no significant changes in perchlorate concentration or uptake (Table 4). In the second field experiment, there was no differences in perchorate content or accumulation in the whole-above ground plant to N rate or source (Table 5). The perchlorate content in the trimmed naked head showed a small decrease with N rate but the differences were small and of no practical importance.

The effects of P fertilization on plant growth and perchlorate uptake in the greenhouse experiment is shown in Table 6. Plant growth, perchlorate concentration, and perchlorate uptake increased to the first increment of P fertilizer. We suspect that this increased perchlorate accumulation may be associated with increased root growth to the first increment of P fertilizer. However, under field conditions, P rate or source had no significant effects on perchlorate accumulation (Table 7). There was a trend for increased perchlorate accumulation with P rate but the effect was not statistically significant.

The effects of ion composition in the Colorado River on perchlorate uptake in a greenhouse study is shown in Table 8. Lettuce irrigated with the water with the highest conductance slightly reduced lettuce growth ($P<0.1$) reduced total perchlorate accumulation by this reduction in growth. However, there were no other significant differences in perchlorate content or accumulation to conductance of irrigation water.

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Table 1. Concentrations and accumulations of perchlorate in the various fractions of iceberg lettuce and in the whole above ground plants during the growing season when irrigated with Colorado River water.

Growth Stage	Plant Part				
	1-4th Outer Leaves	5-8th Outer Leaves	8-12th Outer Leaves	Head	Whole Plant
	Perchlorate Concentration (ug/kg)				
1-2 Leaf	--	--	--	--	170 (28)
6-7 Leaf	110 (14)	28 (13)	--	--	102 (5)
Folding	169 (17)	100 (13)	70 (19)	--	102 (46)
Heading	208 (40)	89 (21)	73 (20)	<MRL	89 (18)
Maturity	108 (18)	79 (5)	46 (14)	<MRL	53 (41)
	Perchlorate Accumulation (g/ha)				
1-2 Leaf	--	--	--	--	0.0005 (0.00006)
6-7 Leaf	0.0160 (0.0025)	0.00087 (0.00040)	--	--	0.0178 (0.0021)
Folding	0.125 (0.016)	0.156 (0.033)	0.100 (0.036)	--	0.42 (0.20)
Heading	0.39 (0.03)	0.30 (0.10)	0.30 (0.07)	<MRL	1.17 (.60)
Maturity	0.66 (0.13)	0.74 (0.02)	0.24 (0.06)	<MRL	2.60 (1.7)

Values in parenthesis show standard deviations.

Table 2. Perchlorate content in whole-above ground iceberg lettuce plants and contents of plants separated into combined frame and wrapper leaves and the edible head during the winter-spring 2002-2003 and fall winter 2003-2004.

Sample	Sample Season	Perchlorate concentration (ug/kg) fresh weight		
		Frame-Wrapper-Leaves	Head	Whole above-ground plant
1	Winter Spring 02-03	94	<MRL	37
2	Winter Spring 02-03	90	<MRL	32
3	Winter Spring 02-03	44	Not Detectable	<MRL
4	Winter Spring 02-03	<MRL	Not Detectable	<MRL
5	Winter Spring 02-03	62	<MRL	27
6	Winter Spring 02-03	<MRL	Not Detectable	<MRL
7	Winter Spring 02-03	42	<MRL	<MRL
8	Winter Spring 02-03	58	Not Detectable	<MRL
9	Winter Spring 02-03	45	Not Detectable	25
10	Winter Spring 02-03	78	<MRL	33
11	Winter Spring 02-03	52	<MRL	28
12	Winter Spring 02-03	48	<MRL	29
13	Winter Spring 02-03	63	<MRL	27
14	Winter Spring 02-03	63	<MRL	<MRL
15	Winter Spring 02-03	39	Not Detectable	27
16	Winter Spring 02-03	77	<MRL	28
17	Winter Spring 02-03	52	<MRL	21
18	Winter Spring 02-03	55	23	30
19	Winter Spring 02-03	43	<MRL	22
20	Winter Spring 02-03	64	<MRL	<MRL
21	Winter Spring 02-03	55	<MRL	<MRL
22	Winter Spring 02-03	56	<MRL	<MRL
23	Winter Spring 02-03	56	25	23
24	Winter Spring 02-03	65	<MRL	22
25	Fall Winter 03-04	109	<MRL	65
26	Fall Winter 03-04	56	<MRL	49
27	Fall Winter 03-04	195	<MRL	142
28	Fall Winter 03-04	59	26	43
29	Fall Winter 03-04	162	<MRL	44
30	Fall Winter 03-04	44	<MRL	23
31	Fall Winter 03-04	74	<MRL	34
32	Fall Winter 03-04	40	<MRL	<MRL
33	Fall Winter 03-04	30	<MRL	<MRL
34	Fall Winter 03-04	121	<MRL	81
35	Fall Winter 03-04	100	<MRL	70
36	Fall Winter 03-04	55	<MRL	49
37	Fall Winter 03-04	55	23	38
38	Fall Winter 03-04	112	23	27

39	Fall Winter 03-04	<MRL	<MRL	<MRL
40	Fall Winter 03-04	58	<MRL	22
41	Fall Winter 03-04	43	<MRL	44
42	Fall Winter 03-04	77	<MRL	34
43	Fall Winter 03-04	55	<MRL	37
44	Fall Winter 03-04	89	<MRL	46

<MRL represents seemingly detectable peak among duplicates and/or replicates but below level that can be quantitated.

Table 3. Perchlorate concentration and accumulation in lettuce as affected by N rate and source in a greenhouse experiment.

N Rate (g/pot)	Above-ground plant			Roots		
	Plant weight (g)	Concentration (ug/kg)	Accumulation (ug/pot)	Root Weight (g)	Concentration (ug/kg)	Accumulation (ug)
0	12.1	731	9.0	7.1	50	0.3
0.37	83.2	311	25.7	21.8	84	1.7
0.70	77.4	341	25.9	18.2	55	1.0
1.40	40.7	184	6.3	10.1	63	0.6
Stat.	L*Q**	L**	L**Q**	L**Q**	NS	Q*
N Source						
Urea	41.9	307	15.6	12.3	67	0.8
NH_4NO_3	80.2	267	22.3	21.3	57	1.2
$Ca(NO_3)_2$	73.6	267	19.8	18.4	87	1.5
$(NH_4)_2SO_4$	72.7	273	19.5	14.9	57	0.9
Stat.	13.7	NS	NS	4.6	NS	NS

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.

Table 4. Soil nitrate, midrib nitrate, marketable yield, N uptake, perchlorate concentration, and perchlorate uptake to N rate in a field experiment.
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N Rate (kg/ha)	Soil Nitrate (mg/kg)	Midrib Nitrate (mg/kg)	Marketable Yield (Mg/ha)	N uptake (kg/ha)	Perchlorate Concentration (ug/kg)	Perchlorate uptake (g/ha)
0	5.9	4556	34.5	94	38	2.8
50	9.3	5889	43.6	93	19	1.4
100	12.2	7556	41.9	102	31	2.6
150	21.7	8444	45.9	160	26	2.4
200	37.2	8222	44.1	121	15	1.2
250	45.1	8111	43.7	139	26	2.2
Stat.	L*Q*	L*Q*	L*Q*	NS	NS	NS

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.

Table 5. Yield and perchlorate contents of trimmed naked lettuce heads and whole above ground plants to N rate and N source in field experiment.

N rate (kg/ha)	Yield (Mg/ha)	Marketable Naked Head		Whole Above-ground plant	
		Perchlorate Concentration (ug/kg)	Perchlorate Accumulation (g/ha)	Perchlorate Concentration (ug/kg)	Perchlorate Accumulation (g/ha)
0	56.5	30.2	1.9	39.7	3.5
100	57.5	26.2	1.6	33.6	3.0
200	58.1	21.6	1.4	35.7	3.2
	NS	L*	L*	NS	NS
N Source					
Urea	58.4	26.4	1.6	36.7	3.4
NH_4NO_3	57.2	21.6	1.3	33.2	2.9
$Ca(NO_3)_2$	56.2	22.5	1.4	33.5	3.0
$(NH_4)_2SO_4$	59.1	25.1	1.6	35.1	3.1
	NS	NS	NS	NS	NS

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.

Table 6. Perchlorate concentration and accumulation in lettuce as affected by P rate in greenhouse experiment.

P Rate (g/pot)	Above-ground			Roots		
	Plant weight (g)	Concentration (ug/kg)	Accumulation (ug/pot)	Root Weight (g)	Concentration (ug/kg)	Accumulation (ug/pot)
0	36.6	232	8.5	10.5	59.4	0.5
0.35	93.7	300	28.1	21.8	47.1	1.0
0.70	97.9	347	32.9	26.1	65.9	1.6
1.40	96.2	360	34.6	20.9	54.8	1.1
Stat.	L**Q**	L**Q*	L**Q**	L**Q**	NS	NS

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.

Table 7. Yield and perchlorate contents of trimmed naked lettuce heads and whole above ground plants to P rate and P source in field experiment.

P rate (kg/ha)	Yield (Mg/ha)	Marketable Naked Head		Whole Above-ground plant	
		Perchlorate Concentration (ug/kg)	Perchlorate Accumulation (g/ha)	Perchlorate Concentration (ug/kg)	Perchlorate Accumulation (g/ha)
0	58.0	22	1.2	24	2.0
50	60.3	37	2.3	34	2.9
100	60.1	33	2.0	50	4.3
150	58.5	26	1.6	57	4.9
Stat.	NS	NS	NS	NS	NS
P Source					
Phosphoric Acid	57.5	29	1.7	48	4.0
Ammonium Polyphosphate	61.8	36	2.2	46	4.1
Stat.	NS	NS	NS	NS	NS

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.

Table 8. Plant weights and perchlorate contents as affected by ion concentration of Colorado River water.

Irrigation Water Conductance (dS/m)	Above-ground Weight (g/pot)	Perchlorate Concentration (ug/kg)	Perchlorate Uptake (ug/pot)
0.18	83.3	335	28.0
0.24	84.0	341	28.2
0.40	85.1	323	27.4
0.61	92.5	316	29.1
1.07	85.8	359	31.0
1.93	76.9	258	19.4
	NS	NS	Q*

*, **, Significant linear (L) or quadratic (Q) trend at the 5% and 1% levels, respectively. NS=not statistically significant.